HORMONAL INDUCTION OF ENZYMES BY COMPUTER SIMULATION AND MODEL SYSTEM ANALYSIS

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SUMMARY

Analog computer analysis of a model system has been performed for tyrosine aminotransferase. Kinetic data reveal that enzyme synthesis simulated on the computer has characteristics similar to experimentally observed phenomena. A functional role is assigned for corticosteroids as well as other hormones involved in enzyme induction. A terminal role is indicated for cyclic AMP in regulation of enzyme synthesis. The proposed model system for induction and regulation of enzyme synthesis has a broad base of generality, which makes it applicable to other inductive hormonal systems.

INTRODUCTION

In a previous publication[1] model systems were presented to explore the mechanisms and modes of hormonal induction of enzymes in the mammalian liver. Of the two models developed, only one will be analyzed here. A second model based on the concept of cytoplasmic repressor[1,2] was not simulated on the computer, since the analysis of the first model on the computer revealed that more positive experimental information is required to confirm the existence of a repressor. Mainly "superinduction" (si) by Actinomycin-D itself is not sufficient evidence to postulate a conceptual repressor. This problem will be dealt with in a more detailed manner in the Discussion.

In order to facilitate the analysis, the scheme of the model (Fig. 1) and Tables 1 and 2 containing symbols and flow equations are presented here from a previous publication.[1] This computer simulation deals primarily with tyrosine aminotransferase (TAT) induction and regulation. However, since the model system was developed on general concepts of hormonal induction of enzymes, the simulation analysis is projected also to some other enzymes. For details of the model, a previous publication[1] should be consulted.

The model system was simulated and a functional system developed on the computer by an empirical approach, using general background information which was available in regard to cellular synthetic processes. For specific detailed procedures, another publication should be consulted.[3]

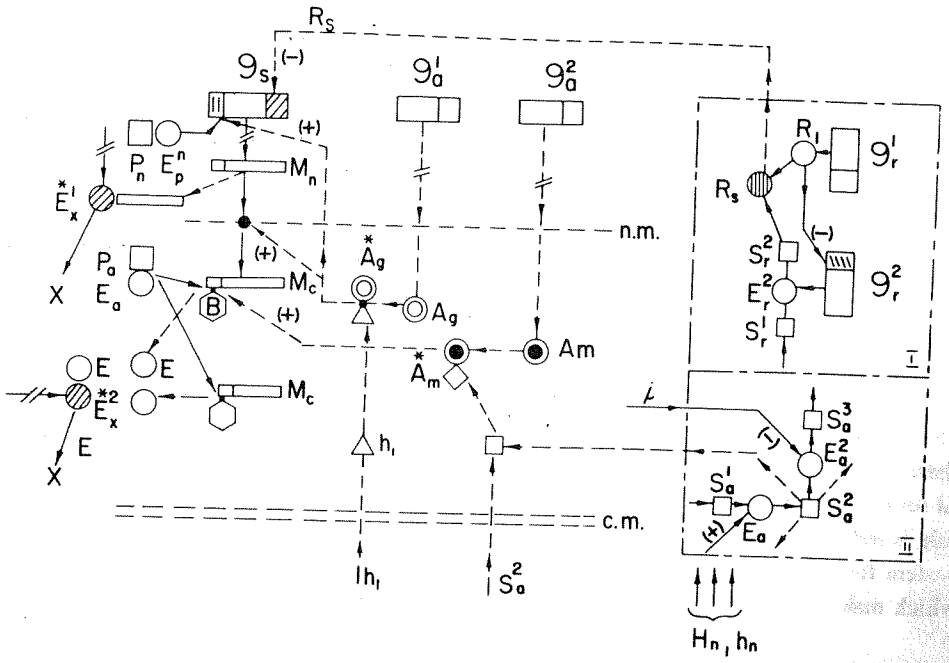

Figure 1. A schematic model system for induction and regulation of enzyme synthesis. [Reprinted from Physiol. Chem. Phys., 2, 351 (1970)].

COMPUTER RESULTS

Basic Functional Entities during the Hormonal Induction of Enzyme Synthesis

Once an operational model was established on the analog computer a variety of experiments could be simulated. Entities were recorded as a function of time (horizontal axis on graphs) and the magnitude of entities is represented on the vertical axis. Hormonal inducers and inhibitors were introduced into the computer simulated model system by using electronic switches (synchronized with computer solution). In Figures 2-6 pulse form was a square wave while in Figures 7-15 pulse was introduced as a slow ramp. In the latter case, slow build-up and decay of pulse simulates more adequately a biological experiment where diffusion and circulation play a role. However, in general both pulse forms are adequate for computer experiments, but square wave pulse produces marked transients in computer solutions and therefore slow ramp is a better way to introduce entities into the system. Both pulse forms are presented in Figure 16. Basic experiments in induction of enzyme (E) by a hormone (h_1) dose is presented in Figure 2. Here basal level of the enzyme is indicated by the curve "O", which remains constant unless an inducer is introduced into the system. Three other curves reveal an increase of enzyme levels as a con-

Table 1. Symbols and Functional Entities for the Model System.

1. G_s - Structural gene for TAT (G_s^* highly active state; it contains a promoter site).
2. G_a - Genes for promoters.
3. G_r - Genes for repressors.
4. M - Messenger RNA for TAT synthesis; M_n (nuclear); M_c (cytoplasmic).
5. A_g - Promoter (protein) activated by h_1.
6. A_g^* - Active promoter operating at transcriptional level.
7. A_m - Promoter activated by S_a^2 (cAMP).
8. A_m^* - Active promoter operating at translational level.
9. P_a - Pool for protein synthesis.
10. P_n - Pool for RNA synthesis; ($P = P_a + P_n$).
11. E_p^n - RNA polymerase.
12. E_a - Amino acid activating enzyme.
13. E_x - Degradative enzymes.
14. E - Induced enzyme TAT
15. h_1 - Corticosteroid.
16. H_n - Hormones (large).
17. h_{1-n} - Various hormones (small).
18. X - Degradation products.
19. R - Repressors.
20. S_r - Substrates in repressor system.
21. E_r - Enzyme in repressor system.
22. E_a - Enzymes in cAMP metabolism.
23. S_a - Substrates in cAMP metabolism.
24. S_a^2 - cAMP (intracellular).
25. i - Inhibitors.
26. B - Ribosomes.
27. (+) - Indicates activation.
28. (-) - Indicates repression.
29. k - Various rate constants.
30. N - Templates.
31. N^* - Highly active template.
32. N_* - Inactive template.
33. n.m. - Nuclear membrane.
34. C.M. - Cell membrane.
35. C - mRNA transport molecule from nucleus to cytoplasm.
36. S_a^{2I} - External cAMP or an analog.

Table 2. Flow Equations for Scheme in Figure 1.

1. $G_s + [E_p P_n] \xrightarrow{k_1} M_n + G_s$
2. $G_s + \overset{*}{A}_g \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \overset{*}{G}_s$
3. $\overset{*}{G}_s + [E_p P_n] \xrightarrow{k_3} M_n + G_s$
4. $G_A^1 + P \xrightarrow{k_4} A_g + G_A^1$
5. $G_A^2 + P \xrightarrow{k_5} A_m + G_A^2$
6. $A_g + h_1 \underset{k_{-6}}{\overset{k_6}{\rightleftharpoons}} \overset{*}{A}_g$
7. $A_m + S_a^2 \underset{k_{-7}}{\overset{k_7}{\rightleftharpoons}} \overset{*}{A}_m$
8. $M_n + C \underset{k_{-8}}{\overset{k_8}{\rightleftharpoons}} [C M_n] \xrightarrow{k_9} M_c$
9. $M_c + B \xrightarrow{k_{10}} N$
10. $N + \overset{*}{A}_m \underset{k_{-11}}{\overset{k_{11}}{\rightleftharpoons}} \overset{*}{N}$
11. $N + [E_a P_a] \xrightarrow{k_{12}} E$
12. $\overset{*}{N} + [E_a P_a] \xrightarrow{k_{13}} E$
13. $E + E_x^2 \xrightarrow{k_{14}} E_x^2 + X$
14. $M_n + E_x^1 \xrightarrow{k_{15}} Ex^1 + X$
15. $M_c + E_x^1 \xrightarrow{k_{16}} E_x^1 + X$
16. $G_s + i_1 \underset{k_{-17}}{\overset{k_{17}}{\rightleftharpoons}} [G_s i_1]$
17. $N + i_2 \underset{k_{-18}}{\overset{k_{18}}{\rightleftharpoons}} [i_2 N]$
18. $N + E_x^1 \xrightarrow{k_{19}} X$
19. $\overset{*}{N} + E_x^1 \xrightarrow{k_{20}} X$
20. $h_1^1 \xrightarrow{k_{21}} h_1$
21. $h_1 \xrightarrow{k_{22}} X$
22. $A_g \xrightarrow{k_{23}} X$
23. $A_m \xrightarrow{k_{24}} X$
24. $S_a^{21} \xrightarrow{k_{25}} S_a^2$
25. $S_a^2 \xrightarrow{k_{26}} X$

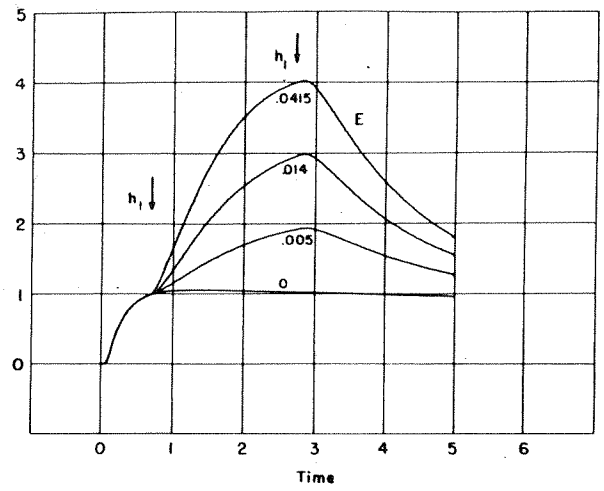

Figure 2. Enzyme (E) synthesis following the introduction of a pulse of corticosteroid (h_1). Numbers on the curves indicate relative concentration levels of hormone. Curve designated by "O" represents basal level of enzyme in the absence of hormone. Arrows indicate the time of introduction (↑) and termination (↓) of hormonal pulse.

sequence of hormonal pulse at 3 different concentrations, but at the same durations. Hormone pulse causes a gradual increase of enzyme concentration, but after the termination of pulse, enzyme level gradually returns to basal level because enzyme E is degraded (k_{14}, Table 2). For the same experiment, Figure 3 shows, in addition to the enzyme, messenger RNA (M_c) and template (N) levels during the pulse induction. These entities also finally decay to the basal level. It is evident that the increase of enzyme synthesis is the result of the additional messenger RNA synthesis where hormone h_1 pulse causes an increase in transcription rate (eq. 3, Table 2).

Experimental observations reveal that glucagon, epinephrine and cyclic-3′,5′-monophosphate (cAMP) are inducing additional TAT synthesis.[4] However, evidence points out that the terminal metabolite in all cases is cAMP and according to our model system it acts here on translational level. Figure 4 shows the effect of cAMP (s_a^2) pulse on enzyme E synthesis. Curve "O" represents ground level enzyme, while relative concentrations of s_a^2 are indicated on curves. It is evident that cAMP induces additional synthesis (eq. 12, Table 2) but enzyme concentration is gradually reduced after the termination of s_a^2 pulse.

Experimental evidence also reveals that corticosteroid (h_1) and glucagon effects are complementary. Namely, when TAT has been induced with corticosteroid in liver, then subsequent induction with glucagon or epinephrine produces an additional increase of enzyme level.[4] It was decided to test the model system performance by simulating aforementioned sequential enzyme induction by two hormones. Glucagon and epinephrine

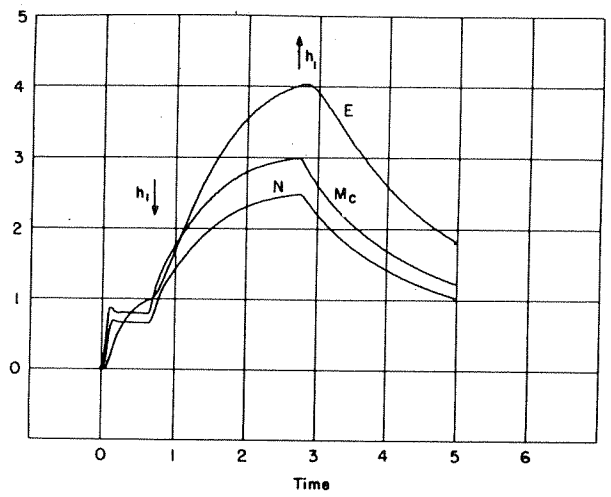

Figure 3. Levels of: enzyme (E), messenger RNA (M_c) and template (N) following the hormonal pulse (h_1).

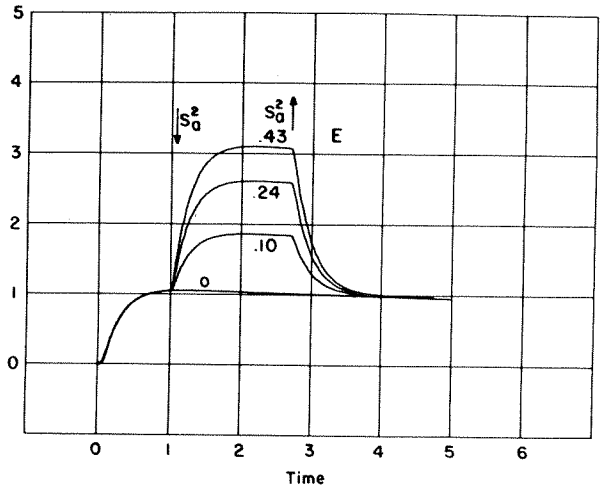

Figure 4. Enzyme (E) synthesis following introduction of pulses of cAMP (s_a^2) at various concentrations.

induction was simulated via the terminal regulatory metabolite cAMP.

The first experiment was performed to test the additive effect of corticosteroid and cAMP in the system. Figure 5 shows the separate and combined effect of two inducers. Rate constants were empirically so adjusted that both inducers produced roughly similar increases in enzyme level. It is evident hormone (h_1) produces the earliest response in enzyme synthesis. This is of course expected since mRNA synthesis is the first step in enzyme synthesis.

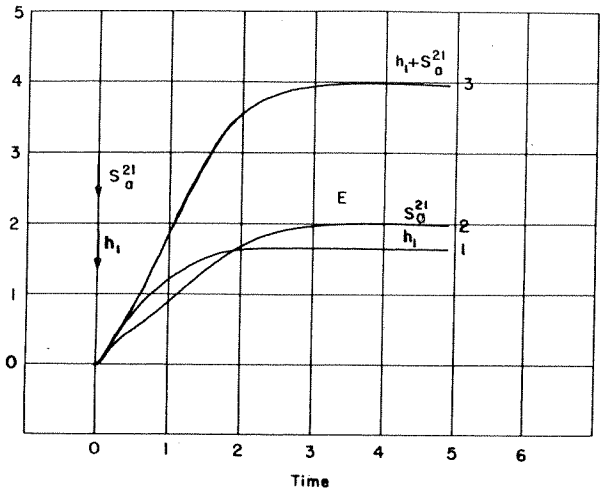

Figure 5. Combined corticosteroid and glucagon effect on enzyme synthesis. Curve "1": Corticosteroid (h_1) alone present in the system. Curve "2": Glucagon (s_a^{21}) alone present in the system. Curve "3": Glucagon and corticosteroid both present in the system.

Figure 6 shows a sequential induction experiment where cAMP pulse is used. Base level enzyme synthesis before the induction is represented by curve "1" ($h_1 = 0$). At the time of the first arrow ($h_1\downarrow$) corticosteroid is introduced into the system and maintained there permanently. Enzyme synthesis increases gradually and reaches finally to a new steady state level (curve 2). At the time of the second arrow ($s_a^2\downarrow$) cAMP is introduced into the system as a pulse. As a consequence an additional enzyme synthesis takes place (curve 3). Enzyme concentration reaches to a steady level and after the termination s_a^2 pulse E concentration returns to the curve "2" level, since h_1 is maintained in the system. It is evident that the increase in enzyme synthesis during sequential induction is the result of increase in the rate of synthesis in both transcriptional and translational processes. It should be noted that some experimental evidence reveals that while corticosteroid induction is long lasting, the glucagon and epinephrine effects are temporary.[4] This phenomen-

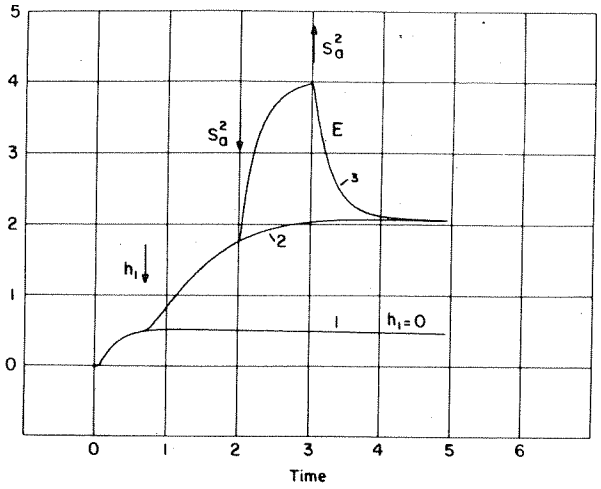

Figure 6. Enzyme (E) synthesis following the introduction of hormone and cAMP into the system. Curve "1": Basal level of E. Curve "2": Hormone h_1 introduced into the system (↓) and maintained there. Curve "3": Conditions as in curve "2", but in addition cAMP pulse introduced.

on will be analyzed in the Discussion section.

Experiments in the literature also show that when corticosteroids are injected into experimental animals and subsequently glucagon or epinephrine is introduced, then the degree of secondary induction depends on the time interval between two sequential injections. The longer the time interval, the smaller the secondary response. It was of great interest to find out whether the current model would yield similar results. Therefore, a time variable sequential induction experiment was simulated on the computer. There hormone h_1 was introduced into the system as a pulse and Figure 7 shows the results. Curve "1" shows the effect of h_1 pulse on additional enzyme synthesis above the base level. In the second and third experiments cAMP was introduced into the system at the first (I) and second (II) arrows respectively. It is indeed evident that the enzyme synthesis level is considerably higher after the first s_a^2 pulse.

Inhibition of Synthetic Processes

It is well known that hormonal induction of enzymes is inhibited by compounds which suppress protein and RNA synthesis.[2,5] Therefore, it was decided to simulate such inhibitory phenomena on computer experiments. Figure 8 shows the Actinomycin-D effect on the level of active gene ($\overset{*}{G}_s$) and template (N). Corticosteroid is continuously present in the system and at the time indicated by the arrow (i_1↓) Actinomycin-D is introduced into the system and maintained there permanently. It is evident that the level of the

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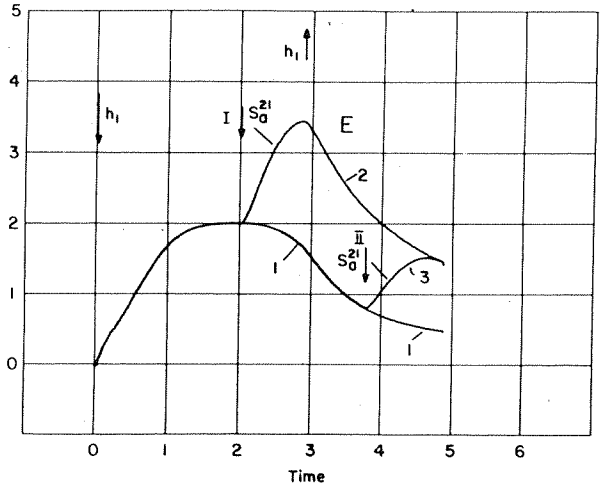

Figure 7. The effect of corticosteroid induction on glucagon induction on enzyme synthesis. Curve "1": Corticosteroid (h_1) pulse alone introduced into the system. Curve "2": Conditions as in "1", but glucagon ($s_a^{2\prime}$) also introduced into the system at the time indicated by first arrow (I↓) and maintained there indefinitely. Curve "3": Conditions as in "2", but glucagon introduced at the time of second arrow (II↓).

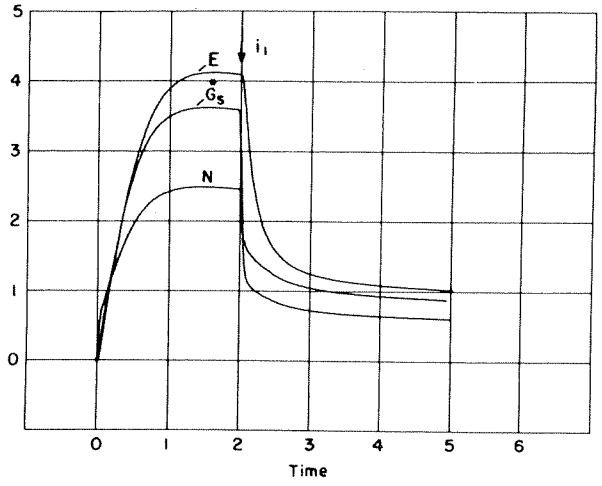

Figure 8. The effect of Actinomycin-D (i_1) on enzyme (E), template (N) and activated gene (G_S^*) levels. Hormone h_1 is maintained continuously in the system, while i_1 is introduced at the time indicated with the arrow (↓) and also maintained indefinitely.

active gene is drastically reduced and converted to an inactive state (eq. 16, Table 2). As a consequence; messenger RNA synthesis is reduced and ribosome and messenger RNA complex (N) establishes itself at a lower steady state level. Enzyme E concentration reveals a similar pattern. It is evident that in such a partial inhibition experiment, all entities have been reduced roughly equally (75%) after the new steady state has been established. Figure 9 reveals the results of a puromycin (i_2) inhibitory experiment at the protein synthesis level. Here the inhibitory process is simulated by inactivating part of

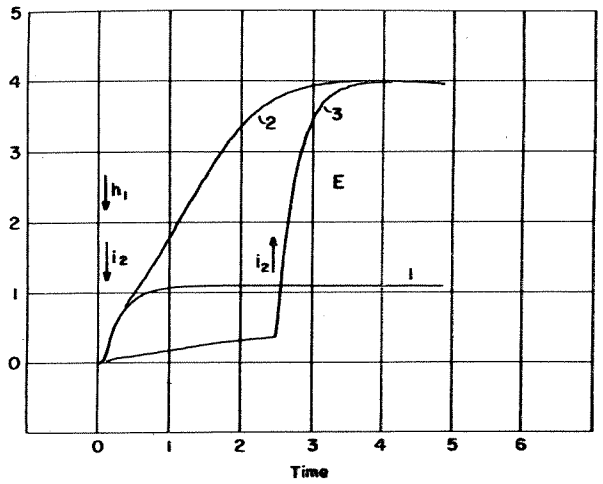

Figure 9. The effect of inhibitors (i_2) of protein synthesis on enzyme induction. Curve "1": Basal level of enzyme. Curve "2": Hormone introduced into the system and maintained indefinitely. Curve "3": Conditions as in curve "2", but in addition pulse of puromycin (i_2) introduced.

template N (eq. 17, Table 2) and converting it into inactive form $[i_2N]$. Curve "1" shows basal level enzyme synthesis in the absence of hormone (h_1). When hormone is introduced into the system with a slow ramp switch, the rate of enzyme E synthesis increases (curve "2") and reaches a new steady state level. However, when the inhibitor (i_2) and hormone (h_1) are introduced simultaneously, enzyme concentration remains far below normal ground state level (curve "1"), but after the removal of inhibitor ($i_2\downarrow$) enzyme synthesis increases rapidly reaching to a normal h_1 induced level. It should be noted that computer simulation experiments are starting from initial conditions where enzyme level is zero and therefore only newly synthesized enzyme is recorded, ignoring the previously existing enzyme. These conditions were selected to avoid long computation times, which are required to establish several steady state conditions starting from initial conditions.

Simulation of Actinomycin-D "Superinducation" (si)

Tomkins et al.[2,5] proposed a general model for TAT induction by corticosteroids, where a basic regulatory role was projected for a cytoplasmic repressor. Principal support for postulating such repressor was derived from Actinomycin-D (AMD) "superinduction" experiments. The essence of "superinduction" is the phenomenon of increased synthesis in cortisone preinduced cells after exposure to AMD. Here the concentration of inhibitor is a critical factor.

In the previous paper in which the basic models for hormonal induction were proposed,[1] we planned to simulate "superinduction" experiments on the computer, based on the Tomkins et al. model. However, during simulation experiments with our current model, we also observed "superinduction" phenomenon in spite of the fact that our model did not contain a cytoplasmic repressor. Therefore, a systematic study was performed as to how the appearance of increased enzyme synthesis after introduction of AMD into the system could be produced. It should be noted that some other interpretations have been reported in the literature for the superinduction phenomenon. Reel and Kenney have reported[6] that Actinomycin-D inhibits degradation of enzyme (TAT). Consequently, higher enzyme levels could be temporarily established after exposure of the experimental system to Actinomycin-D.

Our model system (Fig. 1) was developed to test a variety of interpretations which could be offered to explain "superinduction." A series of experiments were simulated on the computer, and the successful ones are reported here.

1. **Inhibition of messenger RNA (M_m) degradation:** Messenger RNA M_m and M_c for TAT is highly stable[7] and it is degraded by ribonucleases. Here it is considered that Actinomycin-D suppresses the mRNA formation for both enzymes TAT and ribonuclease E_x^{II} which is rapidly turning over.

Computer simulation was carried out as follows: In a cortisone preinduced system Actinomycin-D (i_{II}) was introduced by assigning a value to the rate constant k_{117} via a slow ramp switch. Hormone is maintained in the system permanently. Another ramp switch which was synchronized with the first one, removes E_x^{II} from the system gradually. This mimics the decay of degradative enzyme. Inhibitor concentration effect is simulated by changing the rate constant k_{117} value. Computer experiments revealed that inhibitor (i_{II}) concentration in the model system is indeed a highly critical factor. When i_{II} values are too high or too low, "superinduction" phenomenon is absent. In order to study the phenomenon of "superinduction" as a property of a complex system the degree of superinduction was varied. Figure 10 shows enzyme level at two different k_{117} values (1.0 and 2.0). The effect of i_{II} on TAT alone (E_x^{II} left in the system)) is shown by curve "2" in Figure 11, which can be compared with curve "1" ($k_{117} = 2.0$)). It is obvious that the presence and absence of degrading enzyme E_x^{II} makes the basic difference. It is evident

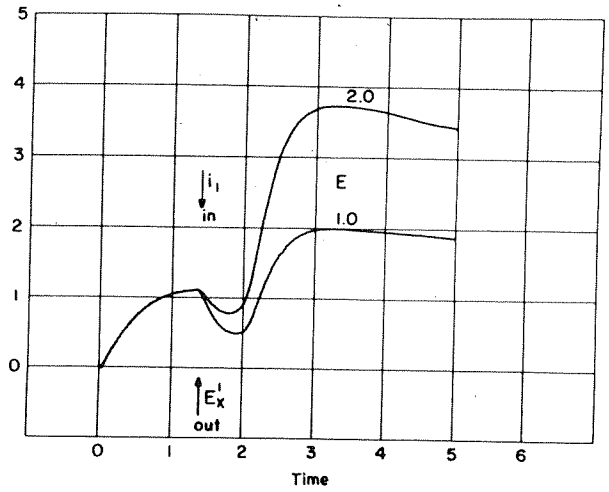

Figure 10. Simulation of Actinomycin-D (AMD) "superinduction" of enzyme E at the level degradation enzyme E_X^1. At the time indicated by arrows AMD (i_1) is introduced into the system and E_X^1 is removed from the system by a slow ramp switch. Enzyme E recorded at two different AMD concentrations. Hormone h_1 present in the system continuously.

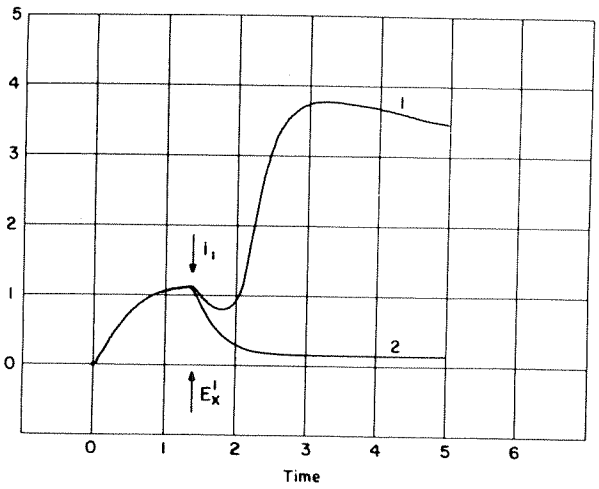

Figure 11. Demonstration of AMD effect at two different conditions. Curve "1": Conditions same as in Figure 10. Curve "2": AMD (i_1) introduced into system as usual, but degradative enzyme E_X^1 maintained in the system.

that by selection of proper rate constant values conditions can be created where increased enzyme (E) takes place. Further experiments were performed to study the effect of protein degrading enzyme E_x^2 on TAT level in the system. For this purpose rate constant k_{14} was varied in a wide range of values. Here at no time could we produce the "superinduction" phenomenon. While the decay rate of enzyme E was modified, its level never increased.

The question was posed as to whether the "superinduction" phenomenon could also be produced in the system in the absence of hormonal inducer. Such base level enzyme "superinduction" has been performed by Tomkins et al. in biological laboratory experiments.[2,5] For convenience in demonstration of such base level "si" in the computer model, the base level mRNA synthesis was slightly increased. We do not go into detail on such adjustments since these are of no great significance. Figure 12 shows that under these conditions, in the absence of hormonal inducer (h_1), the basal "si" can be produced. At k_{17} = 1.0, there is after removal of E_x^1 and introduction of i_1 an initial decay of enzyme E, but it is followed by a temporary increase of enzyme. By doubling the k_{17} value (2.0) "si" phenomenon is readily produced.

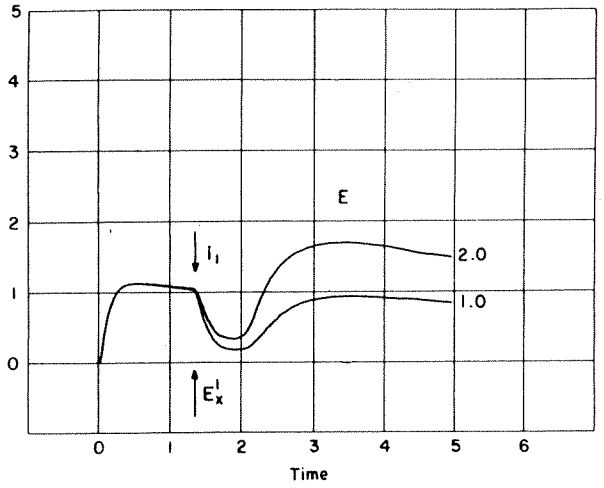

Figure 12. "Superinduction" in the absence of hormone h_1. In order to obtain "superinduction" at the basal level of enzyme synthesis AMD concentration has to be increased above the level used in the experiments with h_1. Experiment carried out as in Figure 10. Relative AMD concentrations indicated on curves.

The effect of degrading enzyme E_x^1 is shown in Figure 13. Curve "1" shows E level when i_1 is present, but k_{15} has a normal value. Curve "2" shows results when k_{15} is removed by a ramp switch.

In all cases, enzyme E level gradually decays to zero level. This is not shown here, since a long time is required on the computer for recording the phenomenon.

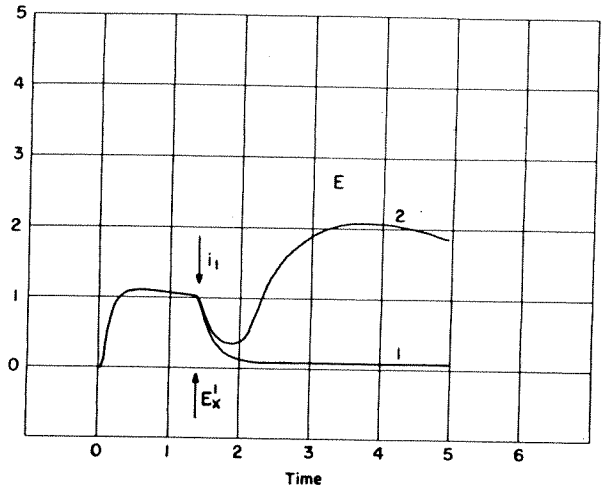

Figure 13. Same experiment as in Figure 12, except in curve "1" E_x^1 is retained in the system, indicating the role of degrading enzyme in "si" phenomenon.

2. **"Superinduction" caused by release of additional ribosomes from nucleoli**: In order to show that "si" phenomenon can be produced by a variety of mechanisms within a complex system, another experiment was organized on the computer. Since Actinomycin-D is a highly toxic compound producing a variety of effects in cells, it was considered that perhaps the release of nucleolar ribosomes on desegregation of ribosomal aggregates could occur.[8] Therefore, the effect of additional release of ribosomes (B) on enzyme E synthesis was simulated on the computer.

To accomplish this the rate constant k_{10} was increased with slow ramp at the same time when i_1 effect was produced by k_{17}. Here hormone h_1 was continuously present in the system. In order to show the quantitative effect of ribosomal release on E synthesis, k_{10} values were varied. Figure 14 shows enzyme E levels at various relative k_{10} values. It is evident that when release of ribosomes is large enough (k_{10} = 4.0 and 5.0) there is indeed a temporary increase in E synthesis, while at lower k_{10} values there is only a delay in enzyme decay. Another experiment was made to study the effect of i_1 concentration

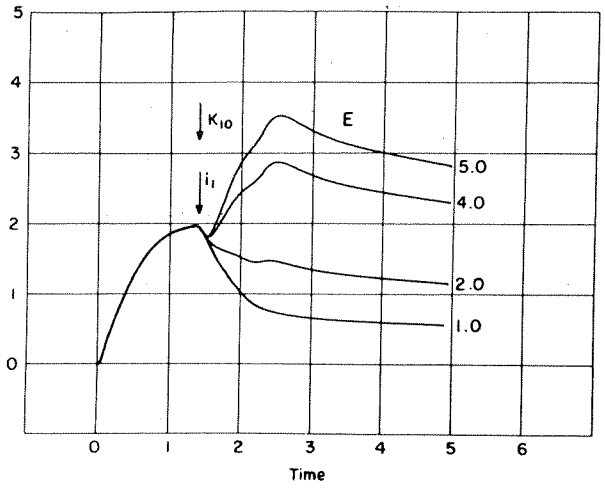

Figure 14. Simulation of "superinduction" by additional release of ribosomes (B) from nucleolus. For simulation purposes, this was accomplished by increasing rate constant k_{10} by a slow ramp indicated by the arrow ($k_{10}\downarrow$) and at the same time i_1 is introduced into the system. Enzyme E concentration is recorded at different k_{10} values. Hormone h_1 is continuously present in the system.

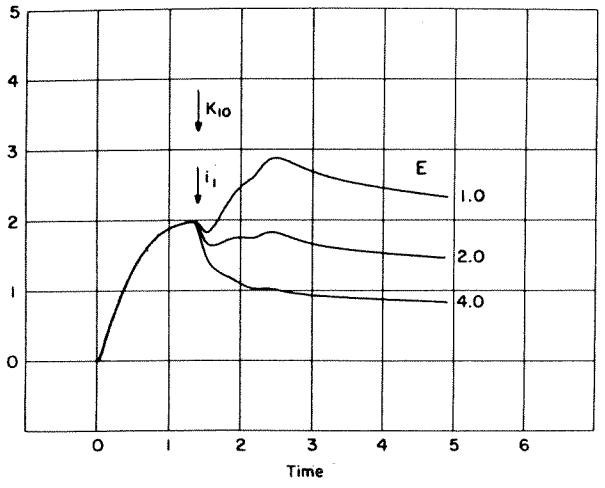

Figure 15. The effect of AMD concentration on enzyme synthesis. Experimental conditions are the same as in Figure 14, where k_{10} = 4.0.

only at the enzyme E level. For this purpose for k_{10} a constant value (4.0) was assigned and rate constant k_{17} was varied. Figure 15 shows the results of such an experiment. It is evident that only at the same intermediate k_{17} values (1.0) is an increase in enzyme synthesis produced, while at high concentrations (k_{17} = 2.0 and 4.0) "si" phenomenon is absent. The same results occur when k_{17} values are small.

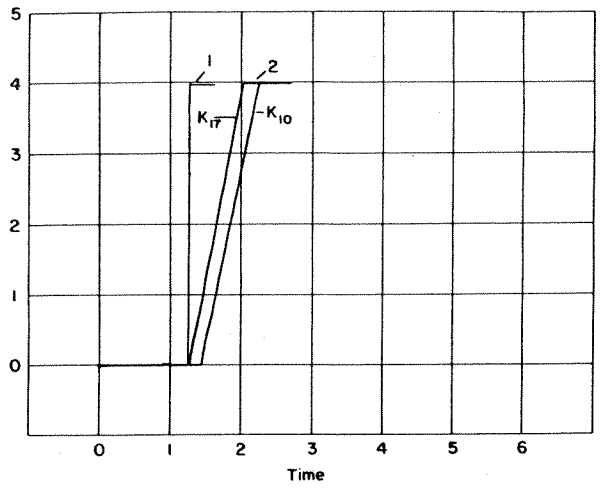

Figure 16. Relative time scale of "pulses" introduced into the system by square pulse (1) or slow ramp switch (2). Note the timing difference between k_{17} (i_1) and k_{10} (B).

DISCUSSION

Kinetic data on enzyme induction by hormones has been obtained from computer simulation experiments. It is evident that time dependent enzyme concentrations exhibit similar patterns which are observed in experimental systems. Furthermore, complementary induction of enzyme (TAT) by corticosteroids and glucagon is clearly demonstrated in computer experiments. Consequently, one can conclude that the proposed model (Fig. 1) serves as an acceptable base to perform future biological experiments and explore the validity of basic concepts on which the model was developed. Especially, the role of hormones in translational and transcriptional processes should be elucidated, including the cAMP role in protein synthesis. Enzyme "superinduction" phenomenon was simulated on the computer by two different mechanisms. This reveals that a complex nonlinear model system can at least conceptually produce "odd" experimental results. In principal,

such phenomenon could occur in real biological systems in selective experimental conditions. Therefore, "si" phenomenon *per se* is not a proof that there is a repressor gene involved in hormonal induction of TAT, but it does not exclude that possibility. However, computer simulated "si" experiments weaken the argument for assuming such a repressor gene.[2] Final verification has to be made experimentally.

While "si" phenomenon may be helpful for developing the model system for hormonal induction of enzymes, one has to consider other factors involved in enzyme synthesis. The model in Figure 1 identifies roles for cAMP and other hormones, and thus a broader generality is displayed. Therefore, it is suggested that the model represented in Figure 1 can serve as a general base to explore the hormonal induction of enzymes. For example, tryptophan pyrolase (TP) induction can be analyzed on the basis of this model system. It is well known that corticosteroids (h_1) are instrumental in enzyme induction, while glucagon and epinephrine are not. However, there is evidence[9] that tryptophan itself acts as a promoter of enzyme synthesis. Therefore, on the basis of the model (Fig. 1), hormone (h_1) would be active as usual at the level of transcription, while tryptophan would have the role displayed by cAMP (s_a^2) in translational processes. As was pointed out previously, glucagon induction of enzyme (TAT) is transient. Repeated dosage of hormone is not effective in increasing enzyme induction as was shown by experiments by Holten and Kenney.[4] It appears that the inductive system is "blocking" itself out after substantial "hormonal stimulus." Such features were not included in the current model (Fig. 1), but will be presented elsewhere,[10] since more experimental information has become available and the phenomenon may be a general feature in some hormonal promotion of enzyme induction.

In TAT induction, cAMP and corticosteroid act in the framework of the model at translational and transcriptional processes respectively. However, there is experimental evidence[11] that in bacteria cAMP can be also active at the level of transcription. Therefore, in the analysis and study of a new hormonal inductive system one has to determine the role of hormonal inducers carefully. If this is done, the basic model in Figure 1 can still be used as a basic framework.

COMMENTS ON THE MODEL SYSTEM

At the time when the model system (Fig. 1) was developed, there was relatively little information available on the role of cAMP in enzyme synthesis. The only source available was the early studies on the enzyme induction in bacteria.[11] However, here also details were lacking in regard to specific mechanisms involved in cAMP action. Therefore, it was necessary to make many postulations in regard to functional entities required to develop the model. It was known that hormonal effects on synthesis occurred at both the translational and transcriptional level, but how the specificity was restricted to a limited number

of enzymes was completely unknown. Therefore, we postulated that there were specific promoter proteins (enzymes) in the cell which were activated by terminal metabolites (for example, cAMP) and those subsequently increased the rate of synthetic processes. The question was posed at that time as to how the promoter protein synthesis was controlled and whether hormones themselves were involved in promoter protein synthesis. This question was left open until further information would become available. In the meantime, receptor proteins for cAMP have been identified in bacteria,[12] which is gratifying, since promoter protein was initially based only on postulations.

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REFERENCES

1. F. Heinmets, *Physiol. Chem. Phys., 2,* 351 (1970).
2. G. M. Tomkins, T. D. Gelehrter, D. Granner, D. Martin, H. H. Samuels and E. B. Thompson, *Science, 166,* 1474 (1969).
3. F. Heinmets, *Quantitative Cellular Biology: An Approach to the Quantitative Analysis of Life Processes,* Marcel Dekker, Inc., New York, 1970.
4. P. Greengard and G. T. Baker, *Science, 154,* 1461 (1966).
   V. Csányi, O. Greengard and E. Knox, *J. Biol. Chem., 242,* 2688 (1967).
   O. Greengard and H. K. Dewey, *J. Biol. Chem., 242,* 2986 (1967).
   D. Holten and F. T. Kenney, *J. Biol. Chem., 242,* 4372 (1967).
   R. J. Wurtman and J. Axelrod, *Proc. Nat. Acad. Sci., 57,* 1594 ((1967).
   T. B. Black and J. Axelrod, *Proc. Nat. Acad. Sci., 61,* 1287 (1968).
   W. D. Wicks, *Science, 160,* 997 (1968).
   O. Greengard, *Science, 163,* 891 (1969).
   O. Greengard, *Biochem. J., 115,* 19 (1969).
5. E. C. C. Lin and W. E. Knox, *Biochim. Biophys. Acta, 26,* 85 (1957).
   F. T. Kenney, *J. Biol. Chem., 237,* 3495 (1962).
   O. Greengard, M. A. Smith and G. Acs, *J. Biol. Chem., 238,* 1548 (1963).
   F. Rosen and C. A. Nichol, *Vitam. Horm., 21,* 135 (1963).
   W. E. Knox and O. Greengard, *Advan. Enzyme Regulation, 3,* 247 (1965).
   E. B. Thompson, G. M. Tomkins and J. F. Curran, *Proc. Nat. Acad. Sci., 56,* 296 (1966).
   G. M. Tomkins, E. B. Thompson, S. Hayashi, T. D. Gelehrter, D. Granner and B. Peterkofsky, *Cold Spring Harbor Symp. Quant. Biol., 31,* 349 (1966).
   T. D. Gelehrter and G. M. Tomkins, *J. Mol. Biol., 29,* 59 (1967).
   D. Martin, G. M. Tomkins and D. Granner, *Proc. Nat. Acad. Sci., 62,* 248 (1969).
6. J. Reel and F. T. Kenney, *Proc. Nat. Acad. Sci., U.S., 61,* 200 (1968).

7. T. B. Levitan and T. E. Webb, *J. Biol. Chem., 244,* 341 (1969).
T. B. Levitan and T. E. Webb, *Biochim. Biophys. Acta, 182,* 491 (1969).
T. B. Levitan and T. E. Webb, *Science, 167,* 283 (1970).
8. T. Staehelin, F. O. Wettstein and H. Noll, *Science, 140,* 180 (1963).
9. W. E. Knox and M. M. Piras, *J. Biol. Chem., 242,* 2959 (1967).
10. F. Heinmets, in press.
11. R. L. Perlman and T. Pastan, *J. Biol. Chem., 243,* 5420 (1968).
T. Pastan and R. L. Perlman, *J. Biol. Chem., 244,* 2226 (1969).
D. A. Chambers and G. Zubay, *Proc. Nat. Acad. Sci., 63,* 118 (1969).
12. T. Pastan and R. Perlman, *Science, 169,* 339 (1970).